A3 *8)* repeating steps *6)* and *7)* from 0 to 119 times, to attach subsequent nucleotide monomers to each of said oligonucleotides produced in step *6)* to produce said plurality of oligonucleotides having a terminal chemically-removable protecting group.

Amendments to the claims are indicated in the attached "Marked Up Version of Amendments" (pages i - iv).

REMARKS

Amendment to the Specification

The related applications paragraph has been added to the specification. Support for the amendment can be found in the Utility Patent Application Transmittal and the Remarks Letter, both of which were filed concurrently with the instant application.

Claim Amendments

Claims 13-42 have been cancelled.

Claims 1 and 3-5 have been amended.

Claim 1 has been amended to recite "an oligonucleotide array comprising a plurality of oligonucleotides." Support for the amendment can be found, for example, at page 2, lines 29-31. Claim 1 has also been amended to recite that protecting groups can additionally be replaced with "2 to 21" phosphate units, so that Claims 10 and 11 are properly dependent from Claim 1.

Claims 3-5 have been amended to provide antecedent basis for the final method step, namely to recite that a nucleotide monomer is attached to an attached monomer.

No new matter has been added.

Status of Claims

Claim 3, along with linking Claims 1, 2, 6-9 and 12 were previously elected for prosecution. The Examiner has requested cancellation of Claims 4, 5, 8-11 and 13-42. However, Claims 2-12 all depend, either directly or indirectly, from Claim 1, a linking claim. As such, Applicants are retaining Claims 1-12, in accordance with MPEP § 809.04:

> Where the requirement for restriction in an application is predicated upon the nonallowability of generic or other type of linking claims, applicant is entitled to retain in the case claims to the nonelected invention or inventions.

Applicants have cancelled Claim 13-42 in this Amendment.

Priority Claim

The Examiner points out that an application that claims the benefit of an earlier application must contain a specific reference to the prior application in the first sentence of the specification or in an application data sheet. Applicants have amended the specification to include a specific reference to the prior application as the first sentence. Applicants believe that no petition under 37 C.F.R. 1.78(a) and no fee under 37 C.F.R. 1.17(t) are required in view of MPEP § 201.11 because the priority claim was made in the application transmittal and the priority claim was recognized by the U.S. Patent and Trademark Office in the filing receipt.

Rejection of Claims 1-3, 6, 7, 9 and 12 Under 35 U.S.C. § 112, Second Paragraph

Claims 1-3, 6,7, 9 and 12 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The Examiner stated that the rejection was made for three reasons, which are addressed separately below.

Claim 1

The Examiner stated that it is not clear whether the objective of the method is to reduce non-specific binding of a target molecule to oligonucleotides located on "designated" or "protected" regions of the surface. The Examiner further stated that it is not clear how introducing a phosphate residue in one specific region will reduce non-specific binding in another region.

Claim 1 has been amended to recite that the claimed method reduces non-specific binding of a target molecule to an oligonucleotide array. It is understood that the oligonucleotide array includes both protected regions of the support and the designated regions. In other words, the claimed method reduces non-specific binding to the solid support as a whole. Thus, Claim 1 as

amended further clarifies that the objective of the method is to reduce non-specific binding of a target molecule to any region of the surface.

Phosphoramidates

The Examiner stated that it is not clear how the use of phosphoramidates will result in the formation of phosphate.

It is well-known in the art that phosphoramidates can readily be reacted to form phosphate groups. Applicants are enclosing herewith sections from "Biochemistry" by R.H. Garrett and C.M. Grisham and "Biochemistry" by D. Voet and J.G. Voet, as Exhibits A and B, respectively. Exhibits A and B each demonstrate that phosphoramidates react with a free hydroxyl group to form a phosphite ester linkage. The phosphite ester is subsequently oxidized (e.g., with iodine) to form a phosphate ester linkage. Applicants note that although the compound labeled as "phosphate-linked bases (dinucleotide)" in Figure 12.32 of Exhibit A is uncharged, the methyl ester can be hydrolyzed by conventional techniques to produce an anionic group (the free acid). In the instant application, the compounds represented by Formulas I and II have a group "X" in the location equivalent to that of the methyl group described above, where "X" is a base-removable protecting group. Thus, following reaction with a free hydroxyl group, the phosphoramidites represented by Formulas I and II are converted into negatively-charged phosphate groups following oxidation and reaction with a base.

It is clear from the teachings of Exhibits A and B that one of skill in the art would immediately recognize that a negatively-charged phosphate residue, as recited in Claim 1, is obtained from a phosphoramidate, such as one recited in Claim 9, by reacting the phosphoramidate with an unprotected hydroxyl group (an activated site from either an oligonucleotide or a protected region) to form a phosphite ester, which is subsequently oxidized and typically deprotected. For this reason, the use of phosphoramidates to produce phosphate is clear to one of skill in the art and no further steps need to be recited.

Claim 3

The Examiner stated that it is not clear whether step b) of Claim 1 is being repeated after each reiteration of step a). The Examiner also stated that Claim 3 lacks internal antecedent basis.

Step a) of Claim 1 involves producing a plurality of oligonucleotides. Step b) of Claim 1 can involve replacing the protecting groups on the plurality of oligonucleotides produced in step a) with a negatively charged phosphate group. It is clear from the use of the word "produced" that step b(i) occurs after step a) has been completed. Moreover, if step b(i) were repeated after each reiteration of step a), the polymer formed would have excess phosphate groups and would not be a conventional oligonucleotide (i.e., one phosphate between ribose or deoxyribose moieties). Thus, one of ordinary skill in the art would clearly recognize that step b) is not repeated after each reiteration of step a).

Claim 3 has been amended to divide former step 3) into new steps 3) and 4). New step 3) involves attaching an independently selected nucleotide monomer having a photolabile protecting group to attached monomers to produce a plurality of oligonucleotides. Step 4) involves repeating step 3) from 0 to 119 times, to attach subsequent nucleotide monomers to each of said oligonucleotides produced in step 3). Thus, step 4) refers back to new step 3) where a monomer is attached to an oligonucleotide, which provides sufficient antecedent basis for step 4).

For the reasons presented above, the claims as amended even more particularly point out and distinctly claim the subject-matter which Applicants regard as the invention. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims 1-3, 6, 7, 9 and 12 Under 35 U.S.C. § 112, First Paragraph

Claims 1-3, 6, 7, 9 and 12 are rejected under 35 U.S.C. § 112, first paragraph, because the Examiner stated that the specification does not reasonably provide enablement for reducing non-specific binding of any unspecified molecule. The Examiner stated that because any molecule present in a biological sample is capable of binding to the surface of an oligonucleotide array, there is a question as to whether the specification provides guidance for the claimed universal effect of reducing non-specific binding.

In making the rejection, the Examiner reasons that any positively charged molecule (e.g., a positively charged protein) would be attracted to the negative charge of the phosphate group, such that non-specific binding would be increased. However, the Examiner's reasoning is incorrect. By virtue of its chemical structure, an oligonucleotide inherently contains a multiplicity of negative charges. If the Examiner's reasoning were correct, the multiple negative

charges of an oligonucleotide would attract a multitude of positively charged molecules, thereby rendering specific binding impossible. This is obviously untrue, as specific binding occurs *in vitro* in the presence of positively charged molecules.

Furthermore, the claimed method involves adding a relatively small number of additional negative charges to an already negatively charged oligonucleotide array; positively charged molecules are not expected to experience a significantly greater non-specific electrostatic attraction to the array. For example, Claims 6-9 recite adding a single negatively charged phosphate to an oligonucleotide of 2-120 monomer units. Similarly, Claims 1-5 and 10-12 recite adding 2 to 21 negative charged phosphate units to the oligonucleotides. In neither case do the additional negatively charged phosphate groups change the overall charge of the oligonucleotide residue.

One key to the present invention is believed to lie in ***reducing*** the number of substances that are attracted to the oligonucleotide array. As stated above, oligonucleotides contain a series of negatively charged phosphate groups. However, protecting groups at the distal end of an oligonucleotide are typically not negatively charged. For instance, common protecting groups for oligonucleotides include the dimethoxytrityl (DMT) and various nitro-substituted aryl groups. Each of these groups are neutral and introduce a large hydrophobic region at the end of an oligonucleotide. Furthermore, the specification at page 12, lines 25-30 teaches that unphotolysed photolyzable protecting groups can react with ethylenediamine in order to produce a positively charged aminoethylcarbamoyl residue at the oligonucleotide surface. ***Neither the hydrophobic protecting groups nor the positively charged aminoethylcarbamoyl residue are found in a "natural" oligonucleotide.*** The fact that the hydrophobic or positively charged groups are on the surface of the oligonucleotide means that a target molecule can readily come into contact with these "unnatural" groups. As such, these "unnatural" groups can cause molecules having hydrophobic or negatively charged regions to non-specifically bind to the oligonucleotide. ***Many of these molecules having hydrophobic or negatively charged regions would not normally be attracted to a negatively-attracted oligonucleotide; thus, protecting groups on an oligonucleotide can induce non-specific binding.*** Thus, in one aspect, the claimed method reduces non-specific binding by eliminating the non-specific binding caused by the presence of a hydrophobic or positively-charged group on the surface of an oligonucleotide.

Many molecules (e.g., proteins) have hydrophobic regions, negatively charged regions, or both. Accordingly, the present method is expected to reduce the non-specific binding attributed to a wide variety of target molecules by eliminating non-specific binding to molecules having hydrophobic regions or negatively charged regions. The Examiner has presented no credible evidence or reasoning to suggest why the examples provided in the present application are not generally applicable. In particular, the Examiner's reasoning regarding positively charged target molecules is shown above to be incorrect. Applicants thus believe that the specification is enabling for the method as claimed. Reconsideration and withdrawal of the rejection are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Jesse C. Fecker

Jesse A. Fecker
Registration No. 52,883
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133
Dated: 5-27-08

MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Please add the below paragraph at page 1, line 5.

RELATED APPLICATION

This application is a continuation of U.S. Application No. 09/063,311, filed April 20, 1998, now abandoned. The entire teachings of the above application are incorporated herein by reference.

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

1. (Amended) A method for reducing non-specific binding of a target molecule to an oligonucleotide array comprising a plurality of oligonucleotides on a surface of a solid support, wherein said surface has a plurality of designated regions and a plurality of protected regions, each of said plurality of protected regions having a protecting group thereon, said method comprising:

a) producing said plurality of oligonucleotides at each of said designated regions, each of said plurality of oligonucleotides having a terminal protecting group; and

b) replacing with a negatively charged phosphate residue or a polyanion chain of 2 to 21 negatively charged phosphate units, at least one of:

i) the protecting groups on each of said plurality of oligonucleotides produced in step *a)*, and

ii) the protecting groups on each of said plurality of protected regions; whereby non-specific binding of said target molecule is reduced.

3. (Amended) The method according to Claim 1, wherein said step *a)* of producing said plurality of oligonucleotides comprises:

1) attaching to each of said designated regions an independently selected linker monomer having a photolabile protecting group;

2) attaching an independently selected nucleotide monomer having a photolabile protecting group to each of said attached linker monomers using light directed methods to produce a plurality of attached monomers each [oligonucleotides] having a terminal photolabile protecting group; [and]

3) attaching an independently selected nucleotide monomer having a photolabile protecting group to each of said attached monomers using light directed methods to produce a plurality of oligonucleotides each having a terminal photolabile protecting group; and

4) repeating step *3)* [*2)*] from 0 to 119 [1 to 120] times, to attach subsequent nucleotide monomers to each of said oligonucleotides produced in step *3)* [*2)*] to produce a plurality of oligonucleotides having a terminal photolabile protecting group.

4. (Amended) The method according to Claim 1, wherein said step *a)* of producing said plurality of oligonucleotides comprises:

1) attaching to each of said designated regions an independently selected linker monomer having a chemically-removable protecting group;

2) replacing each of said chemically-removable protecting groups on each of said attached linker monomers with a photolabile protecting group;

3) attaching an independently selected nucleotide monomer having a chemically-removable protecting group to each of said attached linker monomers using light-directed methods to produce a plurality of attached monomers each [oligonucleotides] having a terminal chemically-removable protecting group;

4) replacing each of said chemically-removable protecting groups on each of said attached monomers [oligonucleotides] with a photolabile protecting group; [and]

5) attaching an independently selected nucleotide monomer having a chemically-removable protecting group to each of said attached monomers using light-directed methods to produce a plurality of oligonucleotides each having a terminal chemically-removable protecting group;

6) replacing each of said chemically-removable protecting groups on each of said oligonucleotides produced in step *5)* with a photolabile protecting group; and

7) repeating steps *5)* [*3)*] and *6)* [*4)*] from 0 to 119 [1 to 120] times, to attach subsequent nucleotide monomers to each of said oligonucleotides produced in step *5)* [3)] to produce said plurality of oligonucleotides having a terminal chemically-removable protecting group.

5. (Amended) The method according to Claim 1, wherein said step *a)* of producing said plurality of oligonucleotides comprises:

1) attaching to each of said designated regions an independently selected linker monomer having a chemically-removable protecting group;

2) forming an activation layer on said designated regions and said protected regions, said activation layer comprising:

i) a photoactive agent, said photoactive agent producing a catalyst when irradiated, and

ii) an autocatalytic agent, said autocatalytic agent generating a product that removes said chemically-removable protecting group when said autocatalytic agent is activated by said catalyst;

3) irradiating a portion of said activation layer overlying said designated regions to remove said chemically-removable protecting group on said linker monomer;

4) attaching an independently selected nucleotide monomer having a chemically-removable protecting group to each of said attached linker monomers, to produce a plurality of attached monomers each [oligonucleotides] having a terminal chemically-removable protecting group;

5) irradiating a portion of said activation layer overlying said designated regions to remove said chemically-removable protecting group on said attached monomers [oligonucleotides];

6) attaching an independently selected nucleotide monomer having a chemically-removable protecting group to each of said attached monomers, to produce a plurality of oligonucleotides each having a terminal chemically-removable protecting group;

7) irradiating a portion of said activation layer overlying said designated regions to remove said chemically-removable protecting group on said oligonucleotides produced in step *6)*; and

8) repeating steps *6)* [*4)*] and *7)* [*5)*] from 0 to 119 [1 to 120] times, to attach subsequent nucleotide monomers to each of said oligonucleotides produced in step *6)* [4)] to produce said plurality of oligonucleotides having a terminal chemically-removable protecting group.

DONALD VOET
University of Pennsylvania

JUDITH G. VOET
Swarthmore College

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D. RNA Sequencing

RNA may be rapidly sequenced by only a slight modification of DNA sequencing procedures. The RNA to be sequenced is transcribed into a complementary strand of DNA (**cDNA**) through the action of **RNA-directed DNA polymerase** (also known as **reverse transcriptase**). This enzyme, which is produced by certain RNA-containing viruses (Section 31-4C), uses an RNA template but is otherwise similar in its action to DNA polymerase I. The resulting cDNA may then be sequenced by either the chemical cleavage or the chain-terminator method.

7. CHEMICAL SYNTHESIS OF OLIGONUCLEOTIDES

Molecular cloning techniques (Section 28-8) have permitted the genetic manipulation of organisms in order to investigate their cellular machinery, change their characteristics, and produce scarce or specifically altered proteins in large quantities. *The ability to chemically synthesize DNA oligonucleotides of specified base sequences is an indispensable part of this powerful technology.* For example, suppose we wished to obtain the gene specifying a protein whose amino acid sequence is at least partially known. Reference to the **genetic code** (the correspondence between an amino acid sequence and the base sequence of the gene specifying it; Section 30-1) permits the synthesis of a short (~15-nucleotide) ^{32}P-labeled oligonucleotide that is complementary to a segment of the gene of interest. The oligonucleotide is used as a probe in the Southern transfer procedure (Section 28-4C) on restriction enzyme-digested DNA from the organism that produced the protein. The probe specifically labels the required gene and thereby permits its isolation.

Synthetic oligonucleotides are also required to specifically alter genes through **site-directed mutagenesis**, a technique pioneered by Michael Smith. An oligonucleotide containing a short gene segment with the desired altered base sequence is used as a primer in the DNA polymerase I replication of the gene of interest. Such a primer will hybridize to the corresponding wild-type sequence if there are only a few mismatched base pairs, and its extension, by DNA polymerase I (Section 28-6C), yields the desired altered gene (Fig. 28-61). The altered gene can then be inserted in a suitable organism via techniques discussed in Section 28-8 and grown (cloned) in quantity.

Oligonucleotides Are Valuable Diagnostic Tools

Synthetic oligonucleotides are widely used as probes in Southern transfer analysis for the diagnosis and prenatal detection of genetic diseases. These diseases often result from a specific change in a single gene such as a base substitution, deletion, or insertion. The temperature at which probe hybridization is carried out may be adjusted so that only an oligonucleotide that is perfectly complementary to a length of DNA will hybridize to it. Even a single base mismatch, under appropriate conditions, will result in a failure to hybridize. For example, sickle-cell anemia arises from a single base change that causes the amino acid substitution Glu $\beta 6 \rightarrow$ Val in hemoglobin (Section 9-3B). A 19-residue oligonucleotide that is complementary to the sickle-cell gene's mutated segment hybridizes, at the proper temperature, to DNA from homozygotes for the sickle-cell gene but not to DNA from normal individuals. An oligonucleotide that is complementary to the normal Hb β gene gives opposite results. DNA from sickle-cell heterozygotes hybridizes to both probes but in reduced amounts relative to the DNAs from homozygotes. The oligonucleotides may consequently be used in the prenatal diagnosis of sickle-cell disease. DNA probes are also rapidly replacing the much slower and less accurate culturing techniques for the identification of pathogenic bacteria.

Oligonucleotides Are Synthesized in a Stepwise Manner

The basic strategy of oligonucleotide synthesis is analogous to that of polypeptide synthesis (Section 6-4): *A suitably protected nucleotide is coupled to the growing end of the*

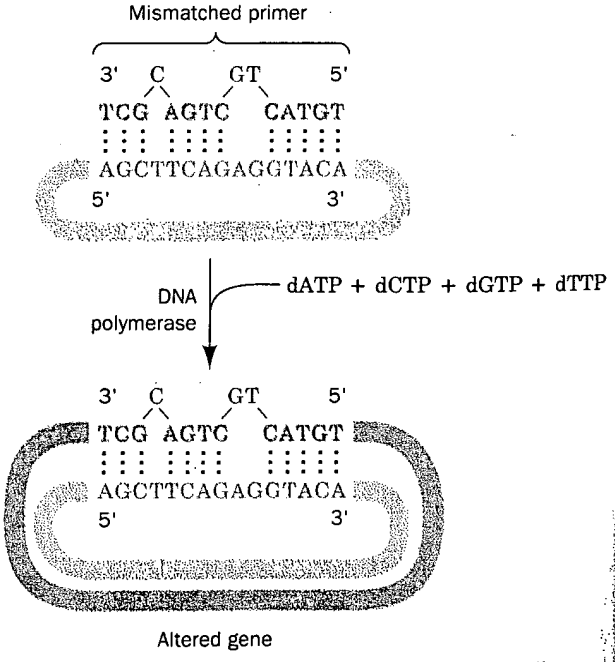

FIGURE 28-61. Site-directed mutagenesis. A chemically synthesized oligonucleotide incorporating the desired base changes is hybridized to the DNA encoding the gene to be altered. The mismatched primer is then extended by DNA polymerase I, thereby generating the mutated gene. The mutated gene can subsequently be inserted into a suitable host organism so as to yield the mutant DNA, or the corresponding RNA, in quantity, produce a specifically altered protein, and/or generate a mutant organism.

oligonucleotide chain, the protecting group is removed, and the process is repeated until the desired oligonucleotide has been synthesized. The first practical technique for DNA synthesis, the **phosphodiester method,** which was developed by H. Gobind Khorana in the 1960s, is a laborious process in which all reactions are carried out in solution and the products must be isolated at each stage of the multistep synthesis. Khorana, nevertheless, used this method, in combination with enzymatic techniques, to synthesize a 126-nucleotide tRNA gene, a project that required several years of intense effort by numerous skilled chemists.

The Phosphoramidite Method

By the early 1980s, these difficult and time-consuming processes had been supplanted by much faster solid phase methodologies that permitted oligonucleotide synthesis to be automated. The presently most widely used chemistry, which was formulated by Robert Letsinger and further developed by Marvin Caruthers, is known as the **phosphoramidite method.** This nonaqueous reaction sequence adds a single nucleotide to a growing oligonucleotide chain as follows (Fig. 28-62):

1. The **dimethoxytrityl (DMTr)** protecting group at the 5′ end of the growing oligonucleotide chain (which is anchored via a linking group at its 3′ end to a solid support, S) is removed by treatment with acid.
2. The newly liberated 5′ end of the oligonucleotide is coupled to the 3′-phosphoramidite derivative of the next deoxynucleoside to be added to the chain. The coupling agent in this reaction is **tetrazole.**
3. Any unreacted 5′ end (the coupling reaction has a yield of over 99%) is capped by acetylation so as to block its extension in subsequent coupling reactions. This prevents the extension of erroneous oligonucleotides.
4. The phosphite triester group resulting from the coupling step is oxidized to the phosphotriester, thereby yielding a chain that has been lengthened by one nucleotide.

This reaction sequence, in commercially available automated synthesizers, can be repeated up to ~150 times with a cycle time of 40 min or less. Once an oligonucleotide of desired sequence has been synthesized, it is released from its support and its various blocking groups, including those on the bases, are removed. The product can then be purified by HPLC and/or gel electrophoresis.

8. MOLECULAR CLONING

A major problem in almost every area of biochemical research is obtaining sufficient quantities of the substance of interest. For example, a 10-L culture of *E. coli* grown to its maximum titer of $\sim 10^{10}$ cells · mL^{-1} contains, at most, 7 mg of DNA polymerase I, and many of its proteins are present in far lesser amounts. Yet, it is rare that as much as half of any protein originally present in an organism can be recovered in pure form. Eukaryotic proteins may be even more difficult to obtain because many eukaryotic tissues, whether acquired from an intact organism or grown in tissue culture, are available in only small quantities. As far as the amount of DNA is concerned, our 10-L *E. coli* culture would contain ~0.1 mg of any 1000-bp length of chromosomal DNA (a length sufficient to contain most prokaryotic genes) but its purification in the presence of the rest of the chromosomal DNA would be an all but impossible task. These difficulties have been largely eliminated in recent years through the development of **molecular cloning** techniques (a **clone** is a collection of identical organisms that are derived from a single ancestor). These methods, which are also referred to as **genetic engineering** and **recombinant DNA** technology, deserve much of the credit for the enormous progress in biochemistry and the dramatic rise of the biotechnology industry since the mid-1970s.

The main idea of molecular cloning is to insert a DNA segment of interest into an autonomously replicating DNA molecule, a so-called ***cloning vector*** or ***vehicle,*** *so that the DNA segment is replicated with the vector.* Cloning such a **chimeric vector** (*chimera:* a monster in Greek mythology that has a lion's head, a goat's body, and a serpent's tail) in a suitable **host organism** such as *E. coli* or yeast results in the production of large amounts of the inserted DNA segment. If a cloned gene is flanked by the properly positioned control sequences for RNA and protein synthesis (Chapters 29 and 30), the host may also produce large quantities of the RNA and protein specified by that gene. The techniques of genetic engineering are outlined in this section.

A. Cloning Vectors

Both plasmids, bacteriophages, and **yeast artificial chromosomes** are used as cloning vectors in genetic engineering.

Plasmid-Based Cloning Vectors

Plasmids are circular DNA duplexes of 1 to 200 kb that contain the requisite genetic machinery, such as a **replication origin** (a site at which DNA replication is initiated; Section 31-2), to permit their autonomous propagation in a bacterial host or in yeast. Plasmids may be considered molecular parasites but in many instances they benefit their host by providing functions, such as resistance to antibiotics, that the host lacks. Indeed, the widespread and alarming appearance, since antibiotics came into use, of antibiotic-resistant pathogens is a result of the rapid proliferation among these organisms of plasmids containing genes that confer resistance to antibiotics.

Some types of plasmids, which are present in one or a few copies per cell, replicate once per cell division as does the bacterial chromosome; their replication is said to be under **stringent control.** The plasmids used in molecular cloning,